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Phytochemical Screening and Solvent Selection for Insecticidal Secondary Metabolites from *Volkameria inermis*, *Senna occidentalis* and *Solanum torvum*

Pooja H. Yadav[1] and Sangeeta A. Godbole[2*]

[1,2]Department of Botany, Jai Hind College, Churchgate, Mumbai, Maharashtra- 400020
*Email : sangeeta.godbole@jaihindcollege.edu.in

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ABSTRACT

Some selective plants, such as *Volkameria inermis*, *Senna occidentalis* and *Solanum torvum*, are known to exhibit pesticidal properties against a range of agricultural pests. These plants contain diverse phytochemicals that contribute to their efficacy. Seven different solvents of varying polarity were analyzed to select the best solvent for extraction of multiple types of pesticidal secondary metabolites. Standard methods of qualitative phytochemical estimation were employed, and the phytochemicals extracted in each solvent were detected. Ethanol as well as water proved to be the best solvent for extraction of maximum types of phytochemicals in all three plants. As alkaloids have strong pesticidal properties specifically against insect pests, a further quantitative estimation of total alkaloid content was performed selectively in ethanolic extracts of leaves of 3 plants using bromocresol green spectrophotometric assay. Ethanolic extract of all three plantsproved to have significant amount of total alkaloid content. This emphasizes the importance of using ethanol as a solvent while preparing a botanical pesticide using combination of above three plants specially targeted towards insect pests susceptible to alkaloids.

Key words : Phytochemical screening, Solvent extraction, Alkaloid content, Insecticidal

INTRODUCTION

Plants often produce phytochemicals that help them adapt to their surroundings. These active substances often exhibit insecticidal properties and help plants in their selected survival even in pest dominated regions. Phytochemicals such as terpenoids, saponins and alkaloids have been known to be detrimental to many categories of insect species (Boate and Abalis, 2020). Phenolics contribute a major class of plant metabolites with specific activity against many microbial organisms and form a major class of phytoalexins. Several kinds of chemicals have been even tested in various combinations to control insect pest (Varma and Borad, 2022). Chemical pesticides are detrimental to our environment as well as human health and often lead to development of resistance within the target insect (Raja, 2013). There are also reports of various chemical fungicides causing potential oncogenic risk (*Siddiqua* et al.,2022). Thus, many researchers explored use of naturally available plant phytochemicals to check their utilization as pest and insect inhibitory agents *(Lengai*, et al., 2020).

Researchers often performed experiments to see the effects of isolated plant metabolites on physiology and behavioral patterns of significant classes of insect pests (*Fischer* et al., 2013). Alkaloids are known to be detrimental to several insect pests. They often interact in synergy with saponins and tannins and act as insect repellent (Adeyemi, 2010; Eich, 2008). Alkaloids function as neurotoxins and attack the acetylcholinesterase receptor site of the insects. This results in alteration of permeability of the neuromuscular junction thus leading to generation of new nerve impulses which lead to spasmodic contractions, delayed larval development, convulsions and death of nymphs as well as adult insects (*Eich*, 2008; *Celis*, et al., 2008). Higher plant species belonging to the Berberidaceae, Amaryllidaceae, Liliaceae, Fabaceae, Papaveraceae, Ranunculaceae, and Solanaceae families are prominently rich in alkaloids (Matsuura and Fett-Neto, 2015). Terpenoids are strong insect repellents as well as feeding deterrents (Junker et al., 2011; *Kortbeek* et al., 2019). Some steroidal terpenoids such as ecdysone from ferns often mimic the insect hormones and thus lead to developmental defects in the insect population (Sahayaraj, 2022). Essential oils with monoterpenes have been reported to have a wide range of antifungal and antibacterial activity. Antifungal activity of essential oil has also been reported against various Post-harvest pathogens (*Siddiqua* et al., 2022). Phenolics play important roles in plant herbivore and pathogen interaction (*Ogbonna* et al., 2016). They exert toxicity upon being ingested by the herbivore insect (*Kortbeek* et al., 2019). Plant tannins are polymers of phenolic compounds and affect insects by reducing the digestibility of food in insect guts. They also cause stomach poisoning and lead to systemic effects in insects (Barbehenn and Constabel, 2011). Flavonoids protect plants by exhibiting larvicidal activity during various stages of insect development (*Morimoto* et al., 2000; *Goławska* et al., 2010;). Saponins are glycosides with soapy characteristics. They affect insects by interacting with cholesterol, disturbing the synthesis of ecdysteroids and thus affecting insect molting and ecdysis (Harmatha, 2000; *Ellen* et al., 2007). The above experiments confirmed that plant phytochemicals can be used to control significant classes of agriculturally important pests and diseases. Since then, a search for novel phytochemicals from varied plant groups continued. We now have a good number of plants already known for their novel metabolites. In the present study, three plants selected with diverse chemical constitution were *Volkameria inermis* L. *Senna occidentalis* (L.) and Solanum torvum Swartz. Volkameria inermis L.belongingto the family Lamiaceae earlier known as *Clerodendrum inerme* (L.) Gaertn., is an evergreen erect bush 3 m tall but sometimes a liana and measures up to 13 m long. It is popularly known as “Seaside Clerodendron" in English (*Manoharan* et al., 2008). It is identified and established that the locally occurring mangrove associate, the so-called *C. inerme* (L.) Gaertn., is *Volkameria inermis* L. (Dutta et al., 2018). It is a rich source of phytochemicals and about 283 compounds, maximally including many different terpenoids derivatives, are reported in many species of genus Volkameria. Flavonoid

glycosides, steroid glycosides as well as cyanogenic glycosides with insecticidal effect were isolated from the same (Wang et al., 2018). *Senna occidentalis* (L.), belonging to the family Fabaceae is a small shrub about 3 ft. in height. It is native to the tropical regions of America and naturalized in Australia, eastern Africa, southern and eastern USA and India. Various types of phytochemicals (alkaloids, phenolics, tannins, terpenoids, saponin, cardiac glycoside, anthraquinones, etc.) have been reported in senna occidentalis and some of them also possess strong antimicrobial activity (*Singh* et al., 2016). Solanum torvum Swartz belonging to the family Solanaceae and commonly known as Turkey Berry is a slim perennial shrub native to India, Central and South America, Brazil, China, and Thailand. The plant is a rich source of phenolic compounds as well as nitrogen containing compounds (*Karmakar* et al., 2015).

This paper summarizes a preliminary study involving qualitative phytochemical estimation of above selected plants carried out to select the best extraction solvent, from 7 assayed solvents with varying polarity. This was to ensure extraction of the maximum variety of phytochemicals already known to be present in each of the three selected plants. This knowledge will be of use while selecting solvents for the preparation of a biopesticide from selected plants to achieve maximum pesticidal properties. As alkaloids contribute to insecticidal properties, an attempt is made to confirm the alkaloid content in each of the 3 plants. Results of above experiment will be used while designing a biopesticide for targeting insect pests that are sensitive to or inhibited selectively by alkaloids.

MATERIALS AND METHODS

Collection of Sample

Fresh aerial parts of three selected plant species were taken from three different locations. Senna occidentalis was taken from lower Parel area in Mumbai, Solanum torvum from Ratnagiri district and Volkameria inermis from Churchgate Mumbai, the plant material was identified and authenticated at St. Xavier's college, Blatter Herbarium, Department of Botany, Mumbai.

Preparation of Extract

Plant material was washed with water and then dried under shade for 10-15 days at RT. After drying, the leaves and stems of each plant were individually ground to form fine powder, sieved using a mesh and then stored in an airtight container for further use. Each plant powder was extracted using seven different solvents (Water, Methanol, Acetone, Ethanol, Ethyl acetate, Benzene and Hexane). For extract preparation, 1gm of sample (dried leaf and stem powder of each selected plant) was separately taken into 10 ml of 7 selected solvents and kept for overnight shaking at RT. After filtering the extracts with Whatman paper no.1 they were centrifuged to obtain clear supernatants constituting the leaf and stem extracts in seven different solvents of selected plants. These were used for qualitative phytochemical analysis. Further, the leaf ethanolic extract of each plant was used for quantitative estimation of alkaloids.

Phytochemical analysis of the extracts

The leaf and stem extracts of all three plants were subjected to preliminary screening of secondary metabolites by standard methods (Harborne, 1973; Shaikh and Patil, 2020).

Test for Phenol (Ferric chloride test)

To 0.5 mL of each solvent extract, 4 drops of 5% $FeCl_3$ were added. A dark blue-green color indicated the presence of phenols (Raaman, 2006; Tiwari et al., 2011).

Test for alkaloids

Dragendorff's test : To 1 mL of extract, 0.5 mL of Dragendorff's reagent was added. A reddish-brown precipitate indicated the presence of alkaloids (De Silva et al., 2017; Singh and Kumar, 2017).

Wagner's test : To 1 ml of each test extract, 0.5 ml of Wagner's reagent was added. A reddish-brown precipitate indicated the presence of alkaloids (Raaman, 2006; *Tiwari* et al., 2011; *Auwal* et al., 2014).

Mayer's test : 1 ml of Mayer's reagent was added to 1 ml of extract. The formation of white precipitation indicated the presence of alkaloids (Raaman, 2006; Singh and Kumar, 2017).

Test for Terpenoids

To 1 ml of plant extract, 1 ml of chloroform was added followed by addition of conc. H_2SO_4. The formation of reddish reddish-brown color at the junction indicates presence of terpenoids (*Gul* et al., 2017).

Test for diterpenoid (Copper acetate test):To 1 ml of extract, 0.5 ml of copper acetate was added. The formation of green color indicated the presence of diterpenes (*Tiwari* et al., 2011; *Pandey* and *Tripathi*, 2014).

Test for Triterpenoids (Salkowski test):To 1 ml of extract, 3 to 4 drops of conc. H_2SO_4 were added, and tubes shaken and then allowed to stand. The formation of yellow color in the lower layer indicated the presence of triterpenoids (*Singh* and *Kumar*, 2017).

Test for Steroids (Libermann-Burchard's test): To 1 ml of extract add 4-5 drops of acetic acid. Further add 3-4 drops of conc. H_2SO_4 from the sides of the test tube and allow it to stand for a while. The formation of a reddish-brown ring at the interface confirmed the presence of steroids (*Jagessar*, 2017).

Test for Tannins (Lead sub acetate test)

To 1 ml plant extract, 4-5 drops of Lead sub acetate solution were added. The formation of creamy white precipitate indicated the presence of Tannins (*Sheel* et al., 2014).

Test for Flavonoids (Alkaline reagent test)

To 1 ml of plant extract, 2 mL of 2% NaOH solution was added. The formation of yellow color which disappears on addition of 5-6 drops of dilute HCL indicated the presence of flavonoids (Audu et al., 2007; Singh and kumar, 2017; *Gul* et al., 2017).

Test for Saponins

1ml of the extract is mixed vigorously with 3 mL of water. The formation of frothing indicated the presence of saponins (Kale, 2020).

Quantitative estimation of Total Alkaloid Content

The total alkaloid content in the ethanolic leaf extract of plants was measured using the Bromocresol Green (BCG) and phosphate buffer test (Shamsa et al., 2008). It involves the reaction of alkaloids with BCG at pH 4.7, which results in a chloroform extractable yellow colored product absorbing at 420 nm. Standard alkaloid Atropine obtained from Yucca laboratories was used as a standard. Solutions containing different concentrations (50 to 300 µg) of atropine were prepared (using 1 mg/ml atropine standard solution), and experimental tubes with plant extracts were analyzed alongside using 0.5 ml of leaf ethanolic extracts of selected plants. These were made to 1ml by adding D/w, mixed with 5 ml BCG solution and 5ml phosphate buffer. The mixture was shaken each time followed by the addition of 2 ml of chloroform. Tubes were vortexed to extract the yellow-colored complex, which was extracted with increasing chloroform additions, followed by vigorous shaking. The chloroform layers were pooled and made to a total volume of 10 ml.The absorbance of the complex in chloroform was measured at 420 nm. Alkaloid content in each sample was determined by the regression equation generated from the atropine calibration curve.

RESULTS AND DISCUSSION

Table.1 : Preliminary qualitative phytochemical analysis of various Polar and Non-Polar extracts of leaf and Stem parts of *Volkameria inermis*

Chemical test	*Volkameria inermis*													
	Leaf							Stem						
	W	ME	AC	ET	EA	Bez	hex	w	ME	AC	ET	EA	Bez	Hex
Phenolics	_	++	+++	++	+	_	_	++	++	++	+	++	_	_
Alkaloids Dragendorff test	++	++	++	++	+	+++	+++	_	++	+++	+++	+++	+++	++
Mayer's test	_	+++	+++	+++	_	_	_	+++	+++	++	+++	_	_	_
Wagner's test	++	++	++	++	++	+++	+++	+++	+	++	+++	+++	+++	+++
Terpenoid test	++	++	+++	+++	++	+++	+++	+++	++	++	+++	+++	++	+++
steroid	+++	++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tri-Terpenoid	+	_	+	+	+	_	+	++	++	++	+	++	_	++
di-terpenoid	+++	+++	+++	+++	+++	++	+	++	++	+	++	+++	_	+
Tannin	+	++	++	++	++	++	+++	++	+++	++	+++	+++	+++	+++
Flavonoids	_	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	++	++
Saponin	_	++	_	+	_	+	+	++	_	_	_	++	+	_

+++: highly present, ++: moderately present, +: Low, -: absent. W: Water, ME: Methanol, AC: Acetone, ET: Ethanol, EA: Ethyl acetate, Bez: Benzene, Hex: Hexane.

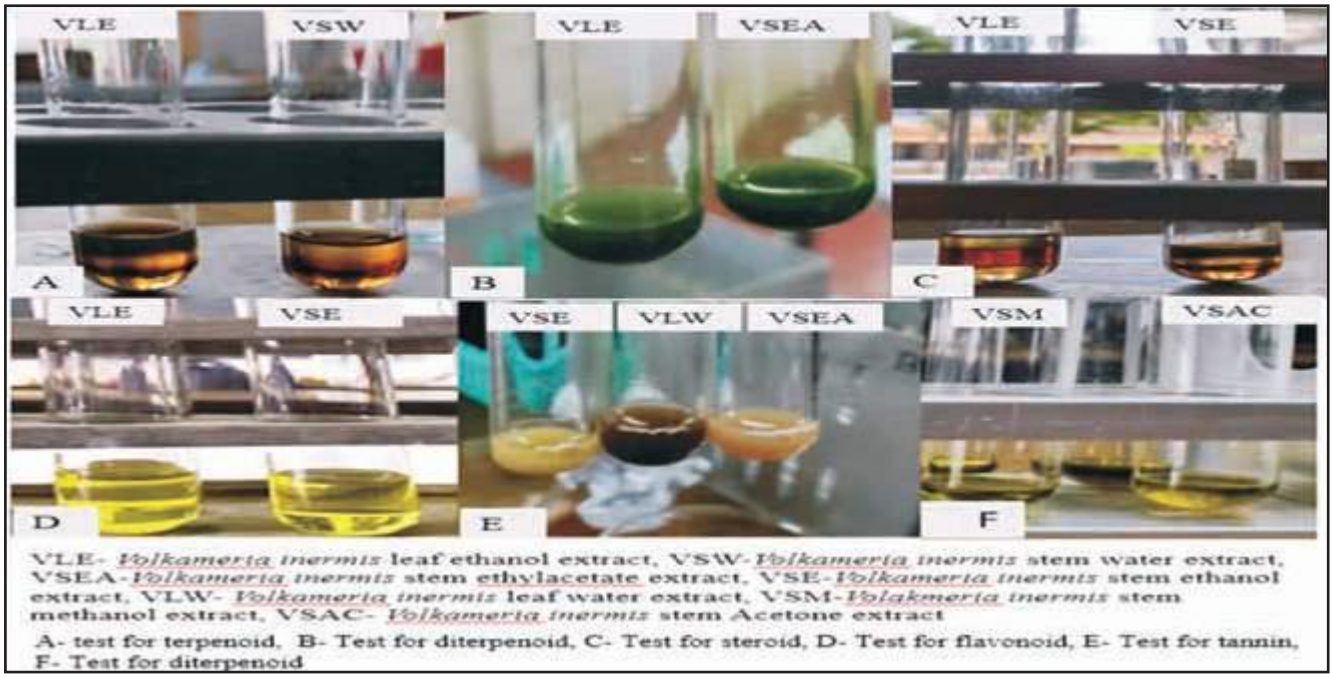

Figure 1 : Phytochemical screening of *Volkameria inermis*

Senna occidentalis:-

Senna occidentalis leaf ethanolic extract showed strong positive results for all tested phytochemical constituents and is clearly a solvent of choice for extracting leaves of *Senna occidentalis* . (Table 2; Figure 2).Ethanolicleaf extract of *S. occidentalis* was earlier reported to show antifeedant activity against *Sitophilus zeamais* Mots (maize weevil) and affects its rate of oviposition (Barau et al., 2020). Methanol leaf extract showed presence of all metabolites except saponins. Methanolic leaf extracts of *S. occidentalis* have earlier been shown to exhibit the greatest antifungal activity against *M. phaseolina which is the* most destructive soil-borne fungal plant pathogens (Javaid et al., 2017). Water leaf extract showed presence of all metabolites except flavonoids. However, these leaf water extracts of *S. occidentalis* were earlier reported to induce complete inhibition of egg hatching and larval development of Haemonchus contortus (*Eguale* et al., 2011).

Water, acetone and ethyl acetate stem extracts showed the presence of all phytoconstituents and should be the preferred solvents for extraction of stem of *Senna occidentalis.* Although alkaloids, steroids, tannins and flavonoids were present in the benzene and n- hexane stem extracts of *Senna occidentalis*, they were devoid of some important insect inhibitory compounds like phenolics and triterpenoids. These results highlight the potential of *Senna occidentalis* as a source of diverse bioactive compounds for insect control.

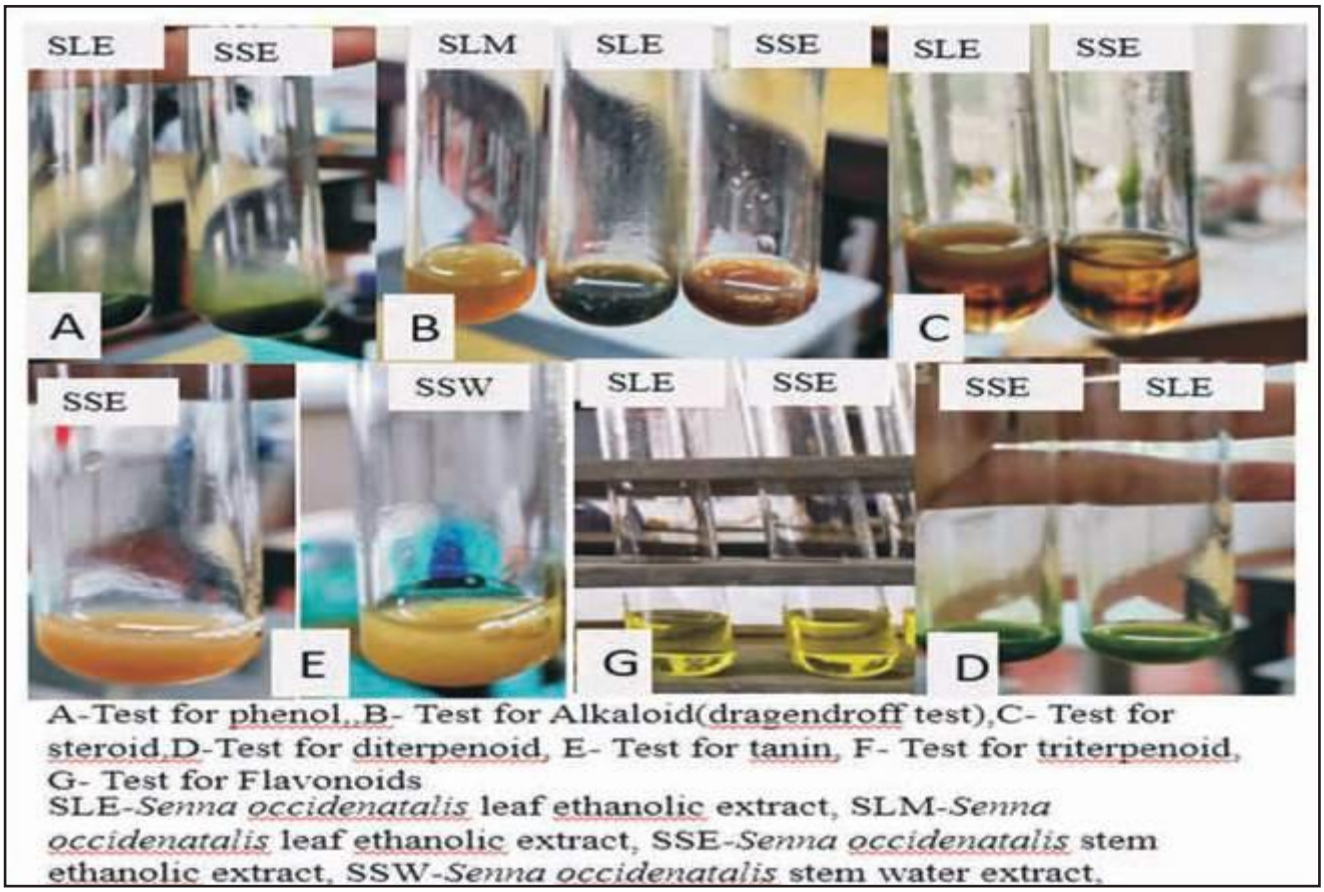

Figure 2 : Phytochemical screening of *Senna occidentalis*

Table 2 : Preliminary qualitative phytochemical analysis of various Polar and Non-Polar extracts of leaf and Stem parts of *Senna occidentalis*

Chemical test	*Senna occidentalis*													
	Leaf							Stem						
	W	ME	AC	ET	EA	Bez	hex	w	ME	AC	ET	EA	Bez	Hex
Phenolics	+	++	+++	+++	+	++	–	++	+++	++	+++	+	–	–
Alkaloids Dragendorff test)	++	+	+	+	+	+++	+++	+++	+++	+++	+++	+	+++	+++
Mayer's test	+	+++	+++	+++	–	–	–	++	+++	+	+++	+	–	–
Wagner's test	+	++	++	+++	++	+++	+++	+++	+++	+++	+++	+	+++	+++
Terpenoid test	+	++	++	++	+	+	++	+++	+++	++	+++	++	+	++
Steroid	+++	+++	+++	+++	+++	++	+	++	+++	++	+++	++	+++	+++
Tri-Terpenoid	+	++	+	++	–	–	–	+	+++	++	++	++	–	–
Di-terpenoid	+++	+++	+++	+++	+++	++	+++	+++	++	+++	+++	++	–	++
Tannin	+++	+	+	++	++	++	++	+++	+++	+++	+++	+++	+++	++
Flavonoids	–	++	+++	+++	+	++	+	+++	+++	+++	+++	+++	++	++
Saponin	+++	–	–	+	–	+++	+	+++	–	+	–	+	+++	–

+++: highly present, ++: moderately present, +: Low, -: absent. W: Water, ME: Methanol, AC: Acetone, ET: Ethanol, EA: Ethyl acetate, Bez: Benzene, Hex: Hexane.

***Solanum torvum*:-**

Ethanol and benzene proved to be the best polar and nonpolar solvent respectively for extraction of phytochemicals from leaves of *Solanum torvum* . (Table 3; Figure 3).Water extracts were also preferred, being just devoid of flavonoids and their extra advantage in being eco-friendly and inexpensive. The leaf water extract of *S. torvum* was earlier reported to exhibit fungitoxic activity against *Fusarium verticillioides, Rhizoctonia solani, Curvularia lunata and Alternaria alternata* (Gupta and Tripathi, 2011). Leaf water extract of *S. torvum* was also reported to inhibit growth of *Colletotrichum musae* Arx causing banana anthracnose disease amongst 25 botanicals tested for activity. Thus, *S. torvum* was earlier suggested as a botanical fungicide alternative to synthetic fungicides to manage fungal disease effectively (*Jadesha* and *Velappagounder*, 2021). Triterpenoids were

absent in methanolic extracts, although five known flavonoids, and 3 alkaloids were reported to be isolated from the EtOAc-soluble fraction of Methanol extract of the whole plant of *S. torvum* (*Tang* et al., 2012). The flavonoid content is suggested to be responsible for its anti-microbial activities (*Shah* et al., 2021). Flavonoids and saponins both were absent in ethyl acetate extracts and phenolics were absent in n-hexane extracts. However, *Solanum torvum* ethyl acetate leaf extract was shown to have maximum repellent and contact toxicity effect, followed by methanol and hexane extract, against adult *Callosobruchus maculatus* (F.) (Murugesan et al.,2021). Thus, while extracting leaves of *Solanum torvum* to prepare a biopesticide against a broad range of insects, based on phytochemical content of plant, ethyl acetate,

Table : 3 Preliminary qualitative phytochemical analysis of various Polar and Non-Polar

Chemical test	*Solanum torvum*													
	Leaf							Stem						
	W	ME	AC	ET	EA	Bez	hex	w	ME	AC	ET	EA	Bez	Hex
Phenolics	+++	+++	+	+++	++	+	_	+++	+	+++	+++	++	_	_
(Alkaloids) Dragendorff test	++	+	+	+	+	+++	+++	+	++	+++	+++	++	+++	+++
Mayer's test	_	+++	+++	+++	+	+	+	++	+++	+	+++	_	_	_
Wagner's test	++	+++	++	++	++	+++	+++	+++	++	+++	+++	++	+++	+++
Terpenoid	+++	+++	+	++	+	++	+++	+++	++	++	++	+	+	_
Steroid	++	++	+++	+++	+++	+	+	++	+++	+++	++	++	++	++
Tri-Terpenoid	+	_	+	++	+	+	+	+++	+	+	+	+	_	_
Di-terpenoid	+++	+++	+++	+++	+++	++	++	+	+++	+++	+++	++	_	++
Tannins	+	++	++	++	+	+	++	++	+++	+++	+++	+++	+++	+++
Flavonoids	_	++	++	+	_	++	+	+++	+++	+++	+++	+++	++	++
Saponin	+++	+++	_	+++	_	++	++	+++	_	_	_	+	++	_

extracts of leaf and Stem parts of *Solanum torvum*

+++: highly present, ++: moderately present, +: Low, -: absent. W: Water, ME: Methanol, AC: Acetone, ET: Ethanol, EA: Ethyl acetate, Bez: Benzene, Hex: Hexane

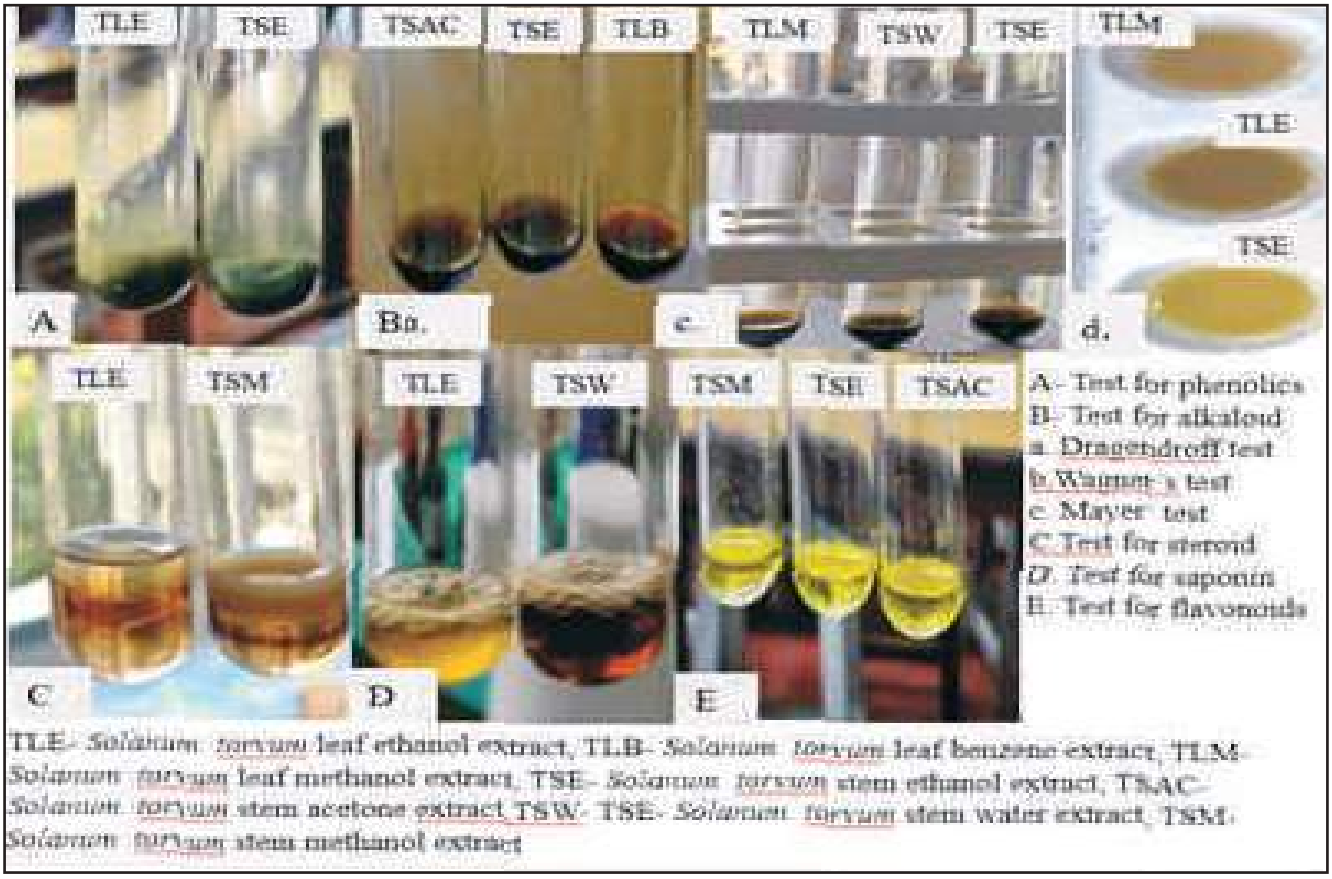

Figure 3 : Phytochemical screening of *Solanum torvum*

The choice of solvent thus significantly influences the efficiency of phytochemical extraction, with ethanol and water emerging as preferred options based on the targeted compounds. Moreover, the accessibility and environmental implications of solvent selection should be considered to ensure sustainable extraction practices. Insect pests vary in their sensitivity towards secondary metabolites occurring in plants. The above results point to the significance of solvent selection for extraction of specific phytochemicals required to combat specific types of insect pests. Overall, these findings contribute to our understanding of plant-based approaches towards pest management and highlight the importance of further research in this area.

Estimation of total Alkaloid Content

The alkaloid content in the leaf ethanolic extracts of three plants was assessed to determine their suitability for biopesticide formulation against alkaloid-sensitive pests. Alkaloids content present was represented as equivalent to standard Atropine (Figure 4). Solanum torvum exhibited the highest alkaloid content followed by Senna occidentalis and Volkameria inermis. (Table 4; Figure 5) This variation highlights the significance of plant selection in biopesticide development, suggesting Solanum torvum as a potential primary ingredient, with Senna occidentalis and Volkameria inermis serving as alternative sources for alkaloid sensitive insects. Solanum torvum contains specific alkaloids like solasonine, solasodine and solamargine which are reported to possess

strong insecticidal activity against Manduca sexta, Tribolium castaneum and Macrosiphum euphorbiae (*Chowański* et al., 2016; Pérez et al., 2007).

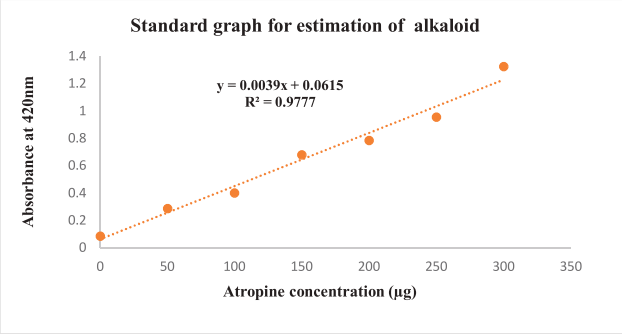

Figure 4. Standard graph for total alkaloid content using Atropine.

The data are mean values of 3 replicates ± SE at 95% confidence (p values < 0.05)

Table 4 : Total Alkaloid content of Ethanolic leaf extracts of Senna occidentalis, Solanum torvum, *Volkameria inermis*.

Plant Samples	Total alkaloid content (mg/gm)
Senna occidentalis	2.351±0.459
Solanum torvum	5.089±0.376
Volkameria inermis	1.417±0.0.091

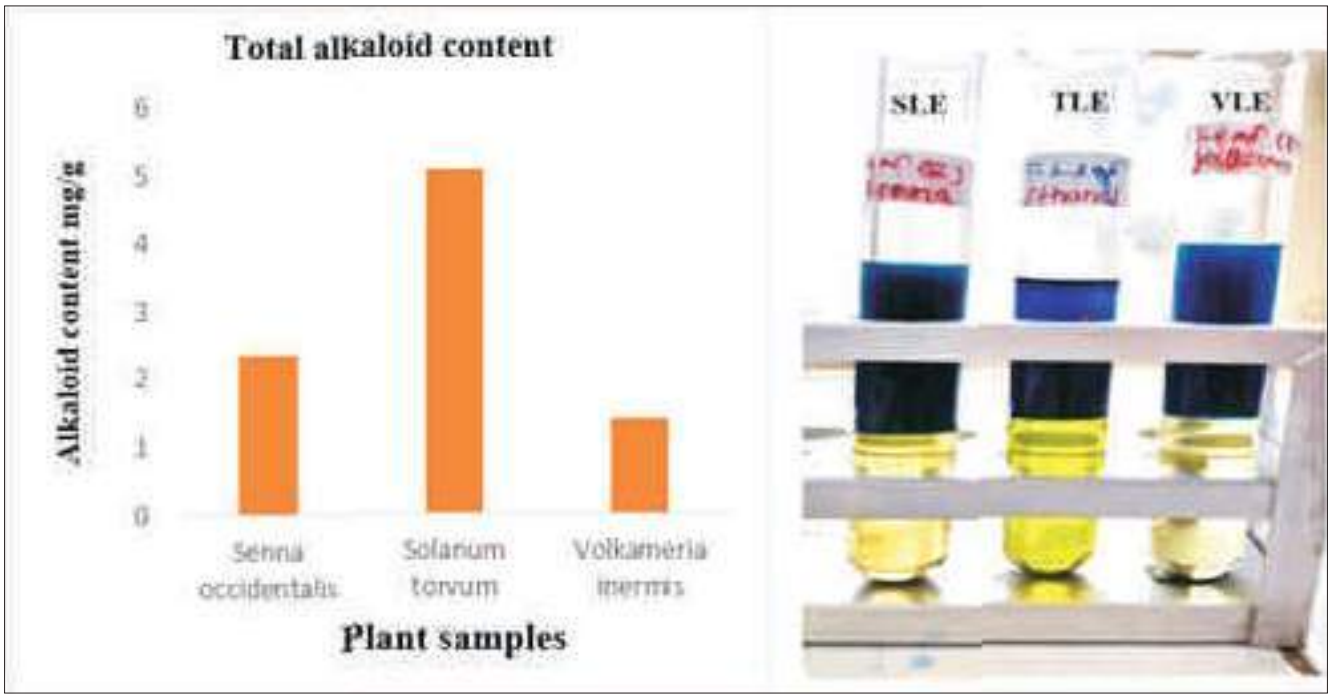

Figure 5. Total alkaloid content (mg /gm of plant extract equivalent to Atropine) in ethanolic leaf extracts of *Senna occidentalis*(SLE), *Solanum torvum* (TLE),*and Volkameria inermis* (VLE).

CONCLUSION

Ethanol and water proved to be effective solvents for extracting multiple types of phytochemicals from *Volkameria inermis* and Solanum torvum, with ethanol preferred for leaf extraction and water for stem extraction in both plants. Hexane and ethyl acetate are

suitable secondary solvents for *Volkameria inermis* leaf and stem, respectively. For *Senna occidentalis*, ethanol is the preferred solvent for leaf extraction, while water, acetone, and ethyl acetate are suitable for stem extraction. Qualitative phytochemical analysis highlighted *Volkameria inermis* to be a good source of terpenoids and Solanum torvum for alkaloids. *Senna occidentalis* gave the best phenolic content followed by alkaloids, terpenoids and tannins as per qualitative phytochemical analysis. The quantitative estimation of alkaloids proved that Solanum torvum had the highest alkaloid content and can be used for designing biopesticides for specific alkaloid sensitive pests. This information can be used to plan biopesticide development against selected or target insect species affected by these major classes of phytochemicals.

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